

WHAT IS CLAIMED IS:

Sub. 7
1 1. An isolated infectious chimeric parainfluenza virus (PIV)
2 comprising a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a large
3 polymerase protein (L), and a partial or complete human parainfluenza virus 3 JS (HPIV3
4 JS) vector genome or antigenome combined with one or more heterologous gene(s) or
5 genome segment(s) encoding one or more antigenic determinant(s) of HN and/or F
6 glycoproteins of HPIV1 and/or HPIV2 to form a chimeric PIV genome or antigenome.

1 2. The chimeric PIV of claim 1, wherein said one or more
2 heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are
3 added adjacent to or within a noncoding region of the partial or complete HPIV3 JS
4 vector genome or antigenome.

1 3. The chimeric PIV of claim 1, wherein said one or more
2 heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are
3 substituted for one or more counterpart gene(s) or genome segment(s) in a partial HPIV3
4 JS vector genome or antigenome.

1 4. The chimeric PIV of claim 1, wherein said one or more antigenic
2 determinant(s) is/are selected from HPIV1 HN and F glycoproteins and antigenic
3 domains, fragments and epitopes thereof.

1 5. The chimeric PIV of claim 4, wherein one or more HPIV1 gene(s)
2 or genome segment(s) encoding one or more HN and/or F glycoprotein(s) or antigenic
3 domain(s), fragment(s) or epitope(s) thereof is/are substituted within the partial or
4 complete HPIV3 JS vector genome or antigenome.

1 6. The chimeric PIV of claim 5, wherein both HPIV1 genes encoding
2 HN and F glycoproteins are substituted for counterpart HPIV3 JS HN and F genes in a
3 partial HPIV3 JS vector genome or antigenome.

1 7. The chimeric PIV of claim 6, wherein the chimeric genome or
2 antigenome incorporates at least one and up to a full complement of attenuating mutations
3 present within HPIV3 JS *cp45* selected from mutations specifying an amino acid
4 substitution in the L protein at a position corresponding to Tyr942, Leu992, or Thr1558 of

5 JS *cp45*; in the N protein at a position corresponding to residues Val96 or Ser389 of JS
6 *cp45*, in the C protein at a position corresponding to Ile96 of JS *cp45*, a nucleotide
7 substitution in a 3' leader sequence of the chimeric virus at a position corresponding to
8 nucleotide 23, 24, 28, or 45 of JS *cp45*, and/or a mutation in an N gene start sequence at a
9 position corresponding to nucleotide 62 of JS *cp45*

1 8. The chimeric PIV of claim 1, wherein one or more HPIV2 gene(s)
2 or genome segment(s) encoding one or more HN and/or F glycoprotein(s) or antigenic
3 domain(s), fragment(s) or epitope(s) thereof is/are added to or incorporated within the
4 partial or complete HPIV3 JS vector genome or antigenome.

1 9. The chimeric PIV of claim 6, wherein a plurality of heterologous
2 genes or genome segments encoding different antigenic determinants of HPIV1 and/or
3 HPIV2 are added to or incorporated within the partial or complete HPIV3 JS vector
4 genome or antigenome.

1 10. The chimeric PIV of claim 9, wherein said plurality of
2 heterologous genes or genome segments encode antigenic determinants from both HPIV1
3 and HPIV2 and are added to or substituted within a partial or complete HPIV3 JS vector
4 genome or antigenome.

1 11. The chimeric PIV of claim 10, wherein one or more HPIV1 gene(s)
2 or genome segment(s) encoding one or more HN and/or F glycoprotein(s) or antigenic
3 domain(s), fragment(s) or epitope(s) thereof and one or more HPIV2 gene(s) or genome
4 segment(s) encoding one or more HN and/or F glycoprotein(s) or antigenic domain(s),
5 fragment(s) or epitope(s) thereof is/are added to or incorporated within the partial or
6 complete HPIV3 JS vector genome or antigenome.

1 12. The chimeric PIV of claim 11, wherein both HPIV1 genes
2 encoding HN and F glycoproteins are substituted for counterpart HPIV3 JS HN and F
3 genes to form a chimeric JS HPIV3-1 vector genome or antigenome which is further
4 modified by addition or incorporation of one or more gene(s) or gene segment(s)
5 encoding one or more antigenic determinant(s) of HPIV2.

1 13. The chimeric PIV of claim 12, wherein a transcription unit
2 comprising an open reading frame (ORF) of an HPIV2 HN gene is added to or
3 incorporated within the chimeric JS HPIV3-1 vector genome or antigenome.

1 14. The chimeric PIV of claim 13 selected from JS rPIV3-1.2HN, or JS
2 rPIV3-1*cp*45.2HN.

1 15. The chimeric PIV of claim 1, wherein the chimeric PIV genome or
2 antigenome is attenuated by addition or incorporation of one gene or cis-acting regulatory
3 element from a bovine PIV3 (BPIV3).

1 16. The chimeric PIV of claim 1, wherein the chimeric PIV genome or
2 antigenome incorporates one or more heterologous, non-coding non-sense polynucleotide
3 sequence(s).

1 17. The chimeric PIV of claim 1, wherein the chimeric genome or
2 antigenome encodes a chimeric glycoprotein having antigenic domains, fragments, or
3 epitopes from both HPIV3 JS and HPIV1 or HPIV2.

1 18. The chimeric PIV of claim 17, wherein the heterologous genome
2 segment encodes a heterologous glycoprotein ectodomain which is substituted for a
3 corresponding glycoprotein ectodomain in the vector genome or antigenome.

1 19. The chimeric PIV of claim 1, wherein the chimeric genome or
2 antigenome is modified by introduction of an attenuating mutation involving an amino
3 acid substitution of phenylalanine at position 456 of the HPIV3 L protein.

1 20. The chimeric PIV of claim 19, wherein phenylalanine at position
2 456 of the HPIV3 L protein is substituted by leucine.

1 21. The chimeric PIV of claim 1, wherein the chimeric genome or
2 antigenome incorporates at least one and up to a full complement of attenuating mutations
3 present within HPIV3 JS *cp*45.

1 22. The chimeric PIV of claim 1, wherein the chimeric genome or
2 antigenome incorporates at least one and up to a full complement of attenuating mutations
3 specifying an amino acid substitution in the L protein at a position corresponding to

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4 Tyr₉₄₂, Leu₉₉₂, or Thr₁₅₅₈ of in JS *cp45*; in the N protein at a position corresponding to
5 residues Val₉₆ or Ser₃₈₉ of JS *cp45*, in the C protein at a position corresponding to Ile₉₆ of
6 JS *cp45*, in the F protein at a position corresponding to residues Ile₄₂₀ or Ala₄₅₀ of JS
7 *cp45*, in the HN protein at a position corresponding to residue Val₃₈₄ of JS *cp45*, a
8 nucleotide substitution in a 3' leader sequence of the chimeric virus at a position
9 corresponding to nucleotide 23, 24, 28, or 45 of JS *cp45*, and/or a mutation in an N gene
10 start sequence at a position corresponding to nucleotide 62 of JS *cp45*.

1 23. The chimeric PIV of claim 21, wherein the chimeric genome or
2 antigenome includes at least one attenuating mutation stabilized by multiple nucleotide
3 changes in a codon specifying the mutation.

1 24. The chimeric PIV of claim 1, wherein the chimeric genome or
2 antigenome incorporates one or more heterologous gene(s) or genome segment(s)
3 encoding one or more respiratory syncytial virus (RSV) F and/or G glycoprotein(s) or
4 immunogenic domain(s), fragment(s), or epitope(s) thereof.

Sub. D1
~~25. The chimeric PIV of claim 1 which is a virus.~~

1 26. The chimeric PIV of claim 1 which is a subviral particle.

1 27. A method for stimulating the immune system of an individual to
2 induce protection against parainfluenza virus (PIV) which comprises administering to the
3 individual an immunologically sufficient amount of the chimeric PIV of claim 1
4 combined with a physiologically acceptable carrier.

1 28. The method of claim 27, wherein the chimeric PIV is administered
2 in a dose of 10^3 to 10^7 PFU.

1 29. The method of claim 27, wherein the chimeric PIV is administered
2 to the upper respiratory tract.

1 30. The method of claim 27, wherein the chimeric PIV is administered
2 by spray, droplet or aerosol.

1 31. The method of claim 27, wherein the chimeric PIV elicits an
2 immune response against one or both of HPIV1 and HPIV2.

1 32. The method of claim 27, wherein the chimeric PIV elicits a
2 polyspecific immune response against multiple HPIVs.

1 33. The method of claim 27, wherein the chimeric PIV and a second
2 recombinant PIV are administered sequentially or simultaneously to elicit a polyspecific
3 immune response.

1 34. An immunogenic composition to elicit an immune response against
2 parainfluenza virus (PIV) comprising an immunogenically sufficient amount of the
3 chimeric PIV of claim 1 in a physiologically acceptable carrier.

1 35. The immunogenic composition of claim 34, formulated in a dose of
2 10^3 to 10^7 PFU.

1 36. The immunogenic composition of claim 34, formulated for
2 administration to the upper respiratory tract by spray, droplet or aerosol.

1 37. The immunogenic composition of claim 34, wherein the chimeric
2 PIV elicits an immune response against one or more virus(es) selected from HPIV1,
3 HPIV2 and HPIV3 JS.

1 38. The immunogenic composition of claim 34, wherein the chimeric
2 PIV elicits an immune response against HPIV3 JS and another virus selected from HPIV1
3 and HPIV2.

5 ab. c3
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1 39. An isolated polynucleotide comprising a chimeric parainfluenza
2 virus (PIV) genome or antigenome which includes a partial or complete human
3 parainfluenza virus 3 JS (HPIV3 JS) vector genome or antigenome combined with one or
4 more heterologous gene(s) or genome segment(s) encoding one or more antigenic
5 determinant(s) of HN and/or F glycoproteins of one or both of HPIV1 and HPIV2 to form
6 a chimeric PIV genome or antigenome.

1 40. The isolated polynucleotide of claim 39, wherein said one or more
2 heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are
3 added adjacent to or within a noncoding region of the partial or complete HPIV3 JS
4 vector genome or antigenome.

1 41. The isolated polynucleotide of claim 39, wherein said one or more
2 heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are
3 substituted for one or more counterpart gene(s) or genome segment(s) in a partial PIV
4 vector genome or antigenome.

1 42. The isolated polynucleotide of claim 39, wherein the chimeric
2 genome or antigenome is attenuated by incorporation of one gene or cis-acting regulatory
3 element from a bovine PIV3 (BPIV3).

1 43. The isolated polynucleotide of claim 39, wherein the chimeric
2 genome or antigenome encodes a chimeric glycoprotein having antigenic domains,
3 fragments, or epitopes from two or more different HPIVs.

1 44. The isolated polynucleotide of claim 39, wherein the chimeric
2 genome or antigenome is further modified by incorporation of an attenuating mutation
3 involving an amino acid substitution of phenylalanine at position 456 of the HPIV3 L
4 protein.

1 45. The isolated polynucleotide of claim 39, wherein phenylalanine at
2 position 456 of the HPIV3 L protein is substituted by leucine.

1 46. The isolated polynucleotide of claim 39, wherein the chimeric
2 genome or antigenome incorporates at least one and up to a full complement of
3 attenuating mutations present within HPIV3 JS *cp45*.

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~~1 47. The isolated polynucleotide of claim 39, wherein the chimeric
2 genome or antigenome incorporates one or more heterologous gene(s) or genome
3 segment(s) encoding one or more respiratory syncytial virus (RSV) F and G
4 glycoprotein(s) or immunogenic domain(s), fragment(s), or epitope(s) thereof~~

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1 48. A method for producing an infectious attenuated chimeric
2 parainfluenza virus (PIV) particle from one or more isolated polynucleotide molecules
3 encoding said PIV, comprising:
4 expressing in a cell or cell-free lysate an expression vector comprising an
5 isolated polynucleotide comprising a partial or complete human parainfluenza virus 3 JS

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6 (HPIV3 JS) vector genome or antigenome combined with one or more heterologous
7 gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of HN
8 and/or F glycoproteins of HPIV1 and/or HPIV2 to form a chimeric PIV genome or
9 antigenome, and PIV N, P, and L proteins.

1 49. The method of claim 48, wherein the chimeric PIV genome or
2 antigenome and the N, P, and L proteins are expressed by two or more different
3 expression vectors.

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1 50. An expression vector comprising an operably linked transcriptional
2 promoter, a polynucleotide sequence which includes a partial or complete human
3 parainfluenza virus 3 JS (HPIV3 JS) vector genome or antigenome combined with one or
4 more heterologous gene(s) or genome segment(s) encoding one or more antigenic
5 determinant(s) of HN and/or F glycoproteins of HPIV1 and/or HPIV2 to form a chimeric
6 PIV genome or antigenome, and a transcriptional terminator.

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